

Appl. No.  
Amdt. dated May 24, 2006  
Preliminary Amendment

PATENT

IAP20 Rec'd PCT/PTO 25 MAY 2006

**Amendments to the Specification:**

Please amend the title as follows:

**"SPECIFIC MARKER Lmx1a ON DOPAMINERGIC NEURONS SPECIFIC TO DOPAMINE-  
PRODUCING NEURON"**

Please amend the paragraph on page 24, lines 7 through 18, beginning,  
"Furthermore, the present invention provides methods for detecting dopaminergic..." as follows:

--Furthermore, the present invention provides methods for detecting dopaminergic neurons, which comprise the step of contacting an anti-Lmx1a antibody with a cellular sample potentially comprising dopaminergic neurons. Specifically, cells expressing an Lmx1a polypeptide and dopaminergic neurons at all differentiation stages, from mature dopaminergic neurons to progenitor cells ~~before proliferation~~ before cell cycle exit, can be detected and selected by contacting cellular samples predicted to comprise dopaminergic neurons or progenitor cells thereof with an antibody of the present invention, and selecting the cells that bind to the antibody. To simplify this detection/selection, the antibodies of the present invention can be labeled or immobilized onto a solid phase. For detection, techniques such as ELISA, RIA, and surface plasmon resonance may be combined. When purification of the selected dopaminergic neurons or progenitor cells thereof is required, the antibodies of the present invention may be used in affinity chromatography.--

Please amend the paragraph on page 24, lines 19 through 22, beginning, "Also, by combining the markers of the present invention with conventional markers that..." as follows:

--Also, by combining the markers of the present invention with conventional markers that detect dopaminergic neurons or progenitor cells thereof, it becomes possible to sort between progenitor cells and mature neurons,

and further to sort between progenitor cells before and after cell cycle exit  
~~division~~--

Please amend the paragraph on page 24, lines 23 through 31, beginning, "For example, progenitor cells and mature neurons can be sorted by combining Lmx1a..." as follows:

--For example, progenitor cells and mature neurons can be sorted by combining Lmx1a with DAT and/or ADH2 as markers. As mentioned above, the Lmx1a gene is confirmed to be widely expressed during differentiation from progenitor cells before cell cycle exit ~~division~~ to mature dopaminergic neurons. On the other hand, as shown in the Examples described below, the DAT and ADH2 genes are expressed after the cells have differentiated into dopaminergic neurons. Therefore, progenitor cells and mature neurons can be separately detected or selected by detecting the expression of an Lmx1a gene using a marker polynucleotide probe or antibody of the present invention and further analyzing the expression of either the DAT gene, the ADH2 gene, or both in cells in which Lmx1a gene expression was detected.--

Please amend the paragraph on page 26, lines 4 through 18, beginning, "Similarly polynucleotides that can detect ADH2 mRNAs are used as the detection..." as follows:

--Similarly polynucleotides that can detect ADH2 mRNAs are used as the detection polynucleotides for detecting ADH2 gene expression based on transcripts. Such polynucleotides for ADH2 detection, which can hybridize to an ADH2 mRNA, comprise the following: (1) DNAs or RNAs comprising a nucleotide sequence complementary to an ADH2 cDNA (SEQ ID NOS: 43 and 45); (2) DNAs or RNAs comprising a nucleotide sequence complementary to a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 44 or 46 and which is a degenerate sequence of the ADH2 ~~DAT~~ gene code; and (3) DNAs or RNAs comprising a nucleotide sequence that can hybridize under stringent conditions to an ADH2 cDNA (SEQ ID NOS: 43 and 45). On the other hand, ADH2-binding antibodies are used to detect ADH2 gene expression at the

translational level. ADH2-binding antibodies comprise antibodies specific to any one of: (1) an ADH2 polypeptide (SEQ ID NOs: 44 and 46); (2) a polypeptide comprising an amino acid sequence with a deletion, insertion, substitution, or addition of one or more amino acids in the amino acid sequence of an ADH2 polypeptide (SEQ ID NOs: 44 and 46) ; or (3) a polypeptide fragment comprising at least six amino acid residues from the polypeptide of (1) or (2).--

Please amend the paragraph on page 26, line 29 through page 27, line 1, beginning, "Next, detection or selection of groups of cells before or after division among the..." as follows:

--Next, detection or selection of groups of cells before or after ~~division~~ cell cycle exit among the progenitor cells can be achieved by combining as markers, (a) the Lmx1a gene and (b) one or more genes selected from the group consisting of Lmx1b, Nurr1, En1, Ptx3, and TH. Lmx1b, Nurr1, En1, Ptx3, and TH are a group of genes expressed in postmitotic precursor cells. Therefore, the expression of these genes can be used to distinguish progenitor cells after cell cycle exit. On the other hand, the Lmx1a gene is also expressed in proliferating progenitor cells before cell cycle exit. Thus, proliferating progenitor cells before cell cycle exit can be detected or selected from among dopaminergic neuron progenitor cells by detecting or selecting cells expressing Lmx1a but do not express Lmx1b, Nurr1, En1, Ptx3, or TH.--

Please amend the paragraph on page 28, line 35 through page 29, line 12, beginning, "Polynucleotides that can detect Ptx3mRNAs are used as detection polynucleotides for..." as follows:

--Polynucleotides that can detect Ptx3mRNAs are used as detection polynucleotides for detecting "Ptx3 gene" expression at the transcriptional level. Such polynucleotides for Ptx3 detection can hybridize to Ptx3mRNAs and comprise the following: (1) DNAs or RNAs consisting of a nucleotide sequence complementary to an Ptx3 cDNA (SEQ ID NOs: 31 and 33); (2) DNAs or RNAs

consisting of a nucleotide sequence complementary to a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 32 or 34 and which is a degenerate sequence of Ptx3 gene code; and (3) DNAs or RNAs consisting of a nucleotide sequence that can hybridize under stringent conditions to a Ptx3 cDNA (SEQ ID NOs: 31 and 33). When detecting ~~ADH2~~ Ptx3 gene expression at the translational level, Ptx3-binding antibodies are used. Ptx3-binding antibodies comprise antibodies that are specific to anyone of: (1) a Ptx3 polypeptide (SEQ ID NOs: 32 and 34); (2) a polypeptide consisting of an amino acid sequence with a deletion, insertion, substitution, or addition of one or more amino acids in the amino acid sequence of a Ptx3 polypeptide (SEQ ID NOs: 32 and 34); or (3) a polypeptide fragment comprising at least six amino acid residues from the polypeptide of (1) or (2).--

Please amend the heading on page 32, line 19, as follows:

--<Analysis of Lmx1a Expression Regulatory Region>--

Please amend the paragraph on page 33, lines 4 through 13, beginning, "The expression region of the Lmx1a gene isolated in this way can be used to produce..." as follows:

--The expression regulatory region of the Lmx1a gene isolated in this way can be used to produce desired polypeptides/proteins *in vivo* in a dopaminergic neuron-specific manner at all developmental stages of the dopaminergic neurons. Also, since Lmx1a is a marker specifically expressed in dopaminergic neurons from a relatively early stage of development, it can also be used to screen dopaminergic neuron differentiation-inducing reagents. Specifically, a vector is first prepared in which a reporter gene which can be detected is introduced under the regulation of the expression regulatory region of the Lmx1a gene, and suitable cells are transformed with this vector. Then, a test substance is contacted with these cells and induction of reporter gene expression by this test substance is detected. When expression of the reporter gene is detected, the test substance is judged to induce the differentiation of dopaminergic neurons.--

Appl. No.  
Amdt. dated May 24, 2006  
Preliminary Amendment

PATENT

Please cancel the present "SEQUENCE LISTING", pages 1/99-99/99, and insert therefor the accompanying paper copy of the Sequence Listing, pages 1-33, at the end of the application.